PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	EFFECT OF TREE NUTS ON METABOLIC SYNDROME CRITERIA: A SYSTEMATIC REVIEW AND META-ANALYSIS OF RANDOMIZED CONTROLLED TRIALS
AUTHORS	Sievenpiper, John; Blanco Mejia, Sonia; Kendall, Cyril; Viguiliouk, Effie; Augustin, Livia; Ha, Vanessa; Cozma, Adrian; Mirrahimi, Arash; Maroleanu, Adriana; Chiavaroli, Laura; Leiter, Lawrence; de Souza, Russell; Jenkins, David

VERSION 1 - REVIEW

REVIEWER	Dr Stephen-Mark Cooper Cardiff Metropolitan University Cardiff Wales, UK
REVIEW RETURNED	03-Mar-2014

GENERAL COMMENTS	I really enjoyed reading the paper and was impressed by the suite of statistics used on the data collected. In this manuscript (MS) the authors apply a set of appropriate statistical tests by way of performing a systematic review and meta-analysis to evaluate the efficacy of tree nuts on MetS criteria in a total of $n = 39$ random control trials that included in $n = 1676$ participants. I am not an expert in nutrition, metabolism or endocrinology nor am I familiar with the bodies of research therein, I have therefore limited myself to making general comments about the MS and its presentation, and more specifically the research design, data collection methods and the statistical analyses of the collected scores. The study is well-designed, the research is well-organised and the data have been collected appropriately. The level of detail provided is very good. As far as the methodology is concerned I am convinced that the study could be replicated based on the description the authors provide in the MS. The authors communicate their arguments in a clear and concise manner in a paper that is well-crafted and is skilfully written. Sometimes there is inconsistency in the expression of terms and units of measurement. The MS could also do with proof reading in terms of spacing. But my specific comments are as follows. I have

used the page numbers and line numbers presumably appended by the authors rather than those generated by the BMJ review software:

<u>Abstract</u> (page 2)

Line 46: and elsewhere throughout the MS … write zero through nine and then 10 through ∞ in the text. So … ≥ three weeks …

Line 52: and extensively elsewhere throughout the MS the authors need to be consistent in spacing … met by 39 RCTs including 1676 …

Line 54: and again … (MD = -0.07 mMol L^{-1} [95%CI: -0.11 through -0.04 mMol L^{-1}]), …

Line 59: and … (MQS < 8). …

<u>Introduction</u> (page 4)

Line 79: I will not comment on this again but the authors need to do a comprehensive proof reading of the MS to deal with the inconsistent spacing … and the …

Line 87: suggest … '… despite a non-significant effect …'

Line 88: and extensively elsewhere throughout the MS … (30 g day^{-1}) …

<u>Methods</u> (page 5)

Line 122: … at least one of the five criteria …

Line 124: … ≥ three weeks …

Line 128: … exclusion was intended to …

Page 6, line 137: … in full by two independent … And on line 138 why do the reviewers need to be identified by their initials? Is this relevant?

Line 159, 160: they are t-ratios and F-ratios and be consistent on P-values or p-values as there is a mixed-economy used in the MS. Note the use of italics.

Page 7, line 166: express them as z-scores (z in italics) but I'm not sure about the ± symbol in mid-sentence. Do you mean … inverse-variance weighting …?

Line 178: all statistical notation in italics … so $P < 0.05$. Why was alpha set at 0.05 in this study? What type of error were the authors trying to avoid?

Line 182: so … $P < 0.10$ and I^2 …

Line 187: this is the BMJ so fibre not fiber! And … < 25 g day^{-1} vs. ≥ 25 g day^{-1} …

Page 8, line 189: … < 50 g day^{-1} vs. ≥ 50 g day^{-1} …

Results (page 8)

Line 208: and elsewhere in the MS all *n* in italics, so *n* = 813 and *n* = 1515 …

Page 9, line 13: the units of measurement are kg so …body mass …

Line 228: and elsewhere throughout the MS … I wonder why there is a need to abbreviate NCEP (for instance) if it is not to be used again in the text? In my view it detracts from reading the MS.

REVIEWER	Ulf Risérus Uppsala university, Sweden
REVIEW RETURNED	14-Mar-2014

GENERAL COMMENTS	The investigators have performed a meta-analysis on the effect of nuts on components of the metabolic syndrome. The data is mainly based on randomized trials, often small and of short duration. Although the results were mainly negative with the exception for triglycerides, the study is timely and relevant given the fact that nuts is a widely recommended food group, and the study adds some knowledge to this issue. The methodology seems appropriate, but there are some issues that need to be clarified. Comments: Did the authors consider to investigate the influence of nut consumption on MetS prevalence? Although only a subset of the study populations are likely to have a full MetS according to given definition, such approach may be informative. The fact that most trials were of low quality could be better discussed, e.g. how did this influence the interpretation and conclusions of this study, and explain if/how this was handled statistically. It seems somewhat difficult to compare different type of study design, i.e. regarding how the nuts were added to the diet, and compared with. For instance using a control group was rare, and studies that added the nuts to the diet might be different in their effects compared with studies that fed them in iso-calorically studies etc. Are the authors confident in that all these type of studies can be combined and have the authors considered to exclude some study type that differed more than other studies in order to have a comparable studies? With this issue in mind, the statement on page 16 (final line) and page 17, line 438-440, might be questioned, i.e. such specific recommendation might not be fully justified.

	It seems from Fig 2 that patients with type 2 diabetes has no apparent benefit of nuts on TG, do the authors have any explanation for this or would like to speculate?
	What is the relevance of TG lowering of 0.07 mmol/l? This is a relevant issue that should be discussed in order to have an idea about the clinical implications of this finding.
	Although fasting insulin is not included as a criteria in the current definition used for MetS, the author could consider adding this component since it may be more relevant than glucose. This both because insulin reflects insulin resistance which is a key feature of the metabolic syndrome, and might well drive the elevated triglycerides and thus influence the effect observed in the present study, but also fasting glucose is less relevant variable in non-diabetic subjects as blood glucose level is tightly regulated and seldom affected by individual dietary components or small dietary changes.
	Page 14, line 374-377. Please slightly develop this part, and add a reference after the sentence on 375-377.
	Page 4. Line 84-85. Is this LDL lowering effect by nuts consistently shown? A reference should be added after that sentence.

VERSION 1 – AUTHOR RESPONSE

REVIEWER 1
Abstract (page 2)
Line 46: and elsewhere throughout the MS … write zero through nine and then 10 through ∞ in the text. So … ≥ three weeks …

AUTHOR REPLY: Thank you for pointing out this issue with the numbering. As requested numbers under 10 have now been spelt out in full. However, we have had to make some exceptions (including "≥ 3 weeks") to remain compliant with BMJ house style: "Numbers under 10 are spelt out, except for measurements with a unit (8 mmol/l) or age (6 weeks old), or when in a list with other numbers (14 dogs, 12 cats, 9 gerbils)."

The following changes were made:

Abstract (page 2)
Line 45: reporting at least one criterion…

Methods (page 5)
Line 121: on at least one of the five criteria of the…
Methods (page 6)
Line 136: extracted in full by two independent…

REVIEWER 1
Line 52: and extensively elsewhere throughout the MS the authors need to be consistent in spacing … met by 39 RCTs including 1676 …

AUTHOR REPLY: As requested, we have adjusted the spacing for consistency throughout. The following changes were made:

Line 299: almond interventions (P < 0.05)
Line 305: HDL-C (MD = 0.00 mmol/L [95% CI, -0.01…
Line 306: heterogeneity (I2 = 86%, P < 0.001)
Lines 316-7: (MD = 0.07 mmHg [95% CI, -1.54… MD = 0.23 mmHg [95% CI, -0.38, 0.83 mmHg])
Line 318: analysis (I2 = 64%, P < 0.001)
Line 323: Appendix Figures 8-A…
Results (page 13)
Line 326-7: (P < 0.05 and P < 0.01 respectively)
Lines 339: (MD = -0.08 mmol/L [95% CI, -0.16, -0.01 mmol/L])… (I2 = 41%, P < 0.05)
Line 348: Appendix Figure 10 presents…
Results (page 14)
Lines 354-5: triglycerides (P < 0.05)

Discussion (page 14)
Line 362: median dose of ~50 g/day…
Lines 364: follow-up of 8 weeks…
Line 374: triglycerides ≥ 1.7mmol/L…
Line 377: diabetes.65 66 This…
Line 378: increasing effect.65 66 Our…
Discussion (page 16)
Line 430: low quality (MQS < 8)
Line 436: available trials were < 3 months…
Discussion (page 17)
Line 449: The median dose of ~50 g/day…

REVIEWER 1
Line 54: and again … (MD = -0.07 mMol L-1 [95%CI: -0.11 through -0.04 mMol L-1]), …

AUTHOR REPLY: As requested, we have adjusted the spacing accordingly:

Abstract (page 2)
Lines 54: MD = -0.06 mmol/L [95% CI, -0.09, -0.03 mmol/L]).
However, SI Units where kept as mmol/L as recommended by the following link provided by the BMJ:
http://www.amamanualofstyle.com/page/si-conversion-calculator

REVIEWER 1
Line 59: and … (MQS < 8). …

AUTHOR REPLY: Thank you for pointing this out. However, the abstract was modified and this line was removed.

REVIEWER 1
Introduction (page 4)
Line 79: I will not comment on this again but the authors need to do a comprehensive proof reading of the MS to deal with the inconsistent spacing … and the …

AUTHOR REPLY: As requested, we have adjusted the spacing accordingly:

Introduction (page 4)

Line 77: and the Food and Drug

REVIEWER 1
Line 87: suggest … '… PubMed despite a non-significant effect …'

AUTHOR REPLY: As requested, we have adjusted the spacing accordingly:

Introduction (page 4)
Line 85: despite a non-significant effect

REVIEWER 1
Line 88: and extensively elsewhere throughout the MS … (30 g day-1) …

AUTHOR REPLY: We apologize for the inconsistency in our units, with some of them expressed as g/d and others as g/day. Thank you for suggesting a different way to abbreviate grams per day, but we would like to keep consistency with the SI units reported in mmol/d. We have, therefore, decided to abbreviate these units as g/day as seen in other BMJ publications (i.e. http://gut.bmj.com/content/early/2014/01/02/gutjnl-2013-304644.full.pdf+html).

To improve consistency, we made the following changes:

Methods (page 8)
Line 194-5: (treatment diet < 25 g/day vs. ≥ 25 g/day
Line 197: tree nut dose (< 50 g/day

Results (page 10)
Line 246: intake was 49.3 g/day… 70.5 g/day)

REVIEWER 1
Methods (page 5)
Line 122: … at least one of the five criteria …

AUTHOR REPLY: As requested, numbers under 10 have been spelt out.

Methods (page 5)
Line 121: at least one of the five criteria…

REVIEWER 1
Line 124: … ≥ three weeks …

AUTHOR REPLY: We agree that numbers under 10 should be spelt out. However, we have not been able to comply here, as this example represents an exception to the rule accordingly to BMJ house style: "Numbers under 10 are spelt out, except for measurements with a unit (8 mmol/l) or age (6 weeks old), or when in a list with other numbers (14 dogs, 12 cats, 9 gerbils)." Therefore, we have not made changes to this suggestion.

REVIEWER 1

Line 128: … exclusion was intended to …

AUTHOR REPLY: We have made the requested change:

Methods (page 5)
Line 127-8: exclusion was intended to…

REVIEWER 1
Page 6, line 137: … in full by two independent … And on line 138 why do the reviewers need to be identified by their initials? Is this relevant?

AUTHOR REPLY: We agree that identifying reviewers by initials may seem unnecessary, but this level of reporting has become the convention for the reporting of systematic reviews and meta-analyses. We have therefore retained the initials.

REVIEWER 1
Line 159, 160: they are t-ratios and F-ratios and be consistent on P-values or p-values as there is a mixed-economy used in the MS. Note the use of italics.

AUTHOR REPLY: As requested, we have used italics in expressing statistical tests such as the F statistics and P-values:

Methods (page 7)
Line 161: values of F-Statistics, and P-values for differences

Results (page 13)
Line 340-1: heterogeneity ($I2 = 41\%$, P-value < 0.05)

REVIEWER 1
Page 7, line 166: express them as z-scores (z in italics) but I'm not sure about the ± symbol in mid-sentence. Do you mean … inverse-variance weighting …?

AUTHOR REPLY: As requested, "Z" has been written in italics, and the ± symbol has been removed:

Methods (page 7)
Line 167-8: were then transformed into z-scores and meta-analyzed…

REVIEWER 1
Line 178: all statistical notation in italics … so P < 0.05.

AUTHOR REPLY: As requested, statistical notations have been written in italics:

Methods (page 7)
Line 178-9: 95% CI and considered significant at P < 0.05.

REVIEWER 1
Why was alpha set at 0.05 in this study? What type of error were the authors trying to avoid?

AUTHOR REPLY: Thank you for this question. We set the alpha at 0.05 to control for type 1 error (i.e. to target a less than 5% "false positive" rate). We were trying to avoid falsely declaring an effect of

nuts on components of the MetS when in fact nuts did not actually have this effect.

REVIEWER 1
Line 182: so … P < 0.10 and I2 …

AUTHOR REPLY: As requested, the statistical notation has been changed. The sentence has also been rewritten for clarity. We have retained the word "quantified" in distinguish the role of the I2 statistic in quantifying the amount of heterogeneity from that of the Cochran Q statistic, which assesses the level of significance. (Methods, Page 7, Lines 182-3)

REVIEWER 1
Line 187: this is the BMJ so fibre not fiber!

AUTHOR REPLY: Our apologies for the spelling oversight. We have corrected the spelling of, fibre throughout:.

Methods (page 8)
Line 194: absolute fibre intake

Appendix Figure 3, Appendix Figure 4, Appendix Figure 6, Appendix Figure 8-A and 8-B, and Appendix Figure 9
We have corrected the word "fibre" in all our subgroup figures for categorical variables, both, within the figure itself and within the legend.

Appendix Table 3.
We have corrected the word "fibre" in each endpoint for fibre intake, change in fibre intake and difference in fibre intake.

REVIEWER 1
And … < 25 g day-1 vs. ≥ 25 g day-1 …

AUTHOR REPLY: We apologize for the inconsistency with the abbreviated units in our manuscript. In order to keep consistency as previously mentioned, we have kept the units as g/day.

REVIEWER 1
Page 8, line 189: … < 50 g day-1 vs. ≥ 50 g day-1 …

AUTHOR REPLY: We apologize for the inconsistency in the abbreviated units in our manuscript. Again, we have kept the units as g/day.

REVIEWER 1
Results (page 8)
Line 208: and elsewhere in the MS all n in italics, so n = 813 and n = 1515 …

AUTHOR REPLY: As requested, we have written "n" in italics for each result:

Results (page 8-9)
Line 213-215: circumference [15 trials, n = 1050], triglycerides [44 trials, n = 1,690], HDL-C [45 trials, n = 2,142], blood pressure [20 trials, n = 1,267], and fasting blood glucose [26 trials, n = 1,360] were included).

REVIEWER 1
Page 9, line 13: the units of measurement are kg so …body mass …

AUTHOR REPLY: We apologize for any confusion. However, it is not clear what the reviewer means with this comment. We have checked all instances in which we reported measurements as kg. In all cases, we did state that the kg were in reference to body weight. Where we provide BMI, results are expressed as kg/m2.

Results (page 9)
Line 226-7: Median body weight for participants was 81.4 kg…

REVIEWER 1
Line 228: and elsewhere throughout the MS … I wonder why there is a need to abbreviate NCEP (for instance) if it is not to be used again in the text? In my view it detracts from reading the MS.

AUTHOR REPLY: As requested, the NCEP abbreviation has been removed:

Results (page 9)
Line 235-6: National Cholesterol Education Program step 1

REVIEWER: 2
Did the authors consider to investigate the influence of nut consumption on MetS prevalence? Although only a subset of the study populations are likely to have a full MetS according to given definition, such approach may be informative.

AUTHOR REPLY: We agree that the effect of tree nut on incident MetS would be informative. Although this was not a pre specified endpoint in our protocol (NCT01630980), it is worth considering. That being said, from the 5 trials (Schutte et al. 2006, Wu et al. 2010, Casas-Agustench et al. 2011, Wang et al. 2012, and Gulati et al. 2014) reporting on participants with confirmed MetS, only one trial (Wu et al. 2010) reported the influence of nut consumption on incident MetS. This randomized study evaluated the incorporation of 30 g of walnuts into lifestyle counseling for a 12 week period. They concluded that even though the prevalence of MetS decreased significantly in both groups, -16.9% for the control arm and -16% for the walnut arm, the reversion rate (proportion of participants who met the criterion at baseline but not at 12 weeks) of 21.1% for the control arm and 25.5% for the walnut arm, was not significantly different between groups. We await more data from trials before we can adequately assess this question.

REVIEWER: 2
The fact that most trials were of low quality could be better discussed, e.g. how did this influence the interpretation and conclusions of this study, and explain if/how this was handled statistically.

AUTHOR REPLY: We agree that the handling of study quality and risk of bias deserves greater attention. Thank you for this point. We did not use these results to adjust our primary statistical meta-analyses. We did, however, perform a priori subgroup analyses for study quality (Heyland MQS < 8 vs. MQS ≥ 8) to assess whether there was a significant interaction with study quality. We did not find that effect of tree nuts differed between low (MQS < 8) and high (MQS ≥ 8) quality trials. Results are shown in appendix figure 3 (for waist circumference), appendix figure 4 (for triglycerides), appendix figure 6 (for high-density lipoprotein), appendixes figures 8-A and 8-B (for systolic and diastolic blood pressure respectively) and appendix figure 9 (for fasting blood glucose). We also provided a description of the extent of low quality scores among the available trials and the main drivers of those scores (lack of double-blinding, loss to follow-up, and poor description of crossovers) in the RESULTS section under Trial Characteristics, page 10, lines 257-9. The Cochrane Risk of Bias tool was used to

add additional information regarding how many of these same quality elements contributed to the risk of bias among the available trials. Although the many domains of the risk of bias (and lack of a single score) precluded subgroup analyses, a description of the extent of high risk of bias among the available trials and the main contributors was provided in the RESULTS section under Trial Characteristics, page 10, lines 259-66. To understand the impact of risk of bias on our results better, we have expanded this description in the narrative. In light of the concerns raised by low quality and risk of bias, we have also added a sentence of caution to the main conclusions: "Careful interpretation of the results is advised, as our conclusions are limited by …".(DISCUSSION, Conclusion, page 17, line 462-3)

This comment also prompted us to explore another source of uncertainty in our analyses. We have run and incorporated sensitivity analyses with correlation values of 0.25, 0.50 and 0.75 to determine whether the overall result of the analyses is robust to the use of a derived pooled correlation coefficients in the paired analyses of crossover trials.

REVIEWER: 2
It seems somewhat difficult to compare different type of study design, i.e. regarding how the nuts were added to the diet, and compared with. For instance using a control group was rare, and studies that added the nuts to the diet might be different in their effects compared with studies that fed them in iso-calorically studies etc. Are the authors confident in that all these type of studies can be combined and have the authors considered to exclude some study type that differed more than other studies in order to have a comparable studies? With this issue in mind, the statement on page 16 (final line) and page 17, line 438-440, might be questioned, i.e. such specific recommendation might not be fully justified.

AUTHOR REPLY: We agree that the included trials incorporated nuts in different ways (i.e. as a snacks, with meals, within bread, as a carbohydrate substitute), and the use of a control group (such as muffin, cheese stick, or pretzel) was rare. However, all included trials were considered isocaloric (this has been addressed in the RESULTS section under Trial Characteristics, page 9-10, lines 240-2), therefore, we believe that the diversity in which the nuts were implemented into diets resembles how people incorporate nuts into their regular diet and supports our statement in the DISCUSSION section, under Practical Implications, page 17, lines 449-51.

REVIEWER: 2
It seems from Fig 2 that patients with type 2 diabetes has no apparent benefit of nuts on TG, do the authors have any explanation for this or would like to speculate?

AUTHOR REPLY: We agree that the data show no apparent TG benefit of tree nuts in people with diabetes. However, we are reluctant to over interpret this finding, as there was no significant interaction for (or effect modification by) health status (P = 0.82). That is, the non-significant TG lowering-effect of tree nuts in people with diabetes (MD = -0.03 mmol/L [95% CI, -0.13, 0.07 mmol/L]) was not statistically different from the significant TG-lowering effect seen in otherwise healthy participants (MD = -0.07 mmol/L [95% CI, -0.11, -0.04 mmol/L)).The same was true for the non-signifcant TG-lowering effects of tree nuts in people with dyslipidemia (MD = -0.06 mmol/L [95% CI, -0.13, 0.00 mmol/L]) or MetS (MD = -0.04 mmol/L [95% CI, -0.13, 0.04 mmol/L]).

REVIEWER: 2
What is the relevance of TG lowering of 0.07 mmol/l? This is a relevant issue that should be discussed in order to have an idea about the clinical implications of this finding.

AUTHOR REPLY: We agree that the clinical relevance that ~50 g/day of tree nuts have on lowering TG at a level of 0.06 mmol/L should be discussed. Given the fact that there is limited evidence to

support any recommendation on a specific target for TG levels, it is hard to assess the clinical significance of this reduction in reducing residual cardiovascular risk. That being said, the reduction in TG can only be considered modest. We have modified the conclusion of our abstract and the main paper accordingly to reflect this qualification "In conclusion, our pooled analyses indicate that daily tree nut consumption has an overall modest metabolic benefit, through a small decrease in triglycerides and fasting blood glucose while preserving waist circumference, HDL-C, and blood pressure in people who are otherwise healthy or have dyslipidemia, criteria of the MetS, or type 2 diabetes." (Conclusion, page 17, lines 456-9)

REVIEWER: 2
Although fasting insulin is not included as a criteria in the current definition used for MetS, the author could consider adding this component since it may be more relevant than glucose. This both because insulin reflects insulin resistance which is a key feature of the metabolic syndrome, and might well drive the elevated triglycerides and thus influence the effect observed in the present study, but also fasting glucose is less relevant variable in non-diabetic subjects as blood glucose level is tightly regulated and seldom affected by individual dietary components or small dietary changes.

AUTHOR REPLY: We agree that fasting insulin is an important marker. We did not include this and other markers of glycemic control in the present review, as it is not part of the definition used for MetS, and, as such, we did not pre specify fasting insulin as an endpoint in our protocol (NCT01630980). That being said, we do have an ongoing systematic review and meta-analysis on the effect of tree nuts on glycemic control, for which we did pre specify fasting insulin as an endpoint. Please stay tuned!

REVIEWER: 2
Page 14, line 374-377. Please slightly develop this part, and add a reference after the sentence on 375-377.

AUTHOR REPLY: As requested, we have developed this part and added a reference to the suggested sentence. Changes are in the DISCUSSION section, under results in relation to other studies, page 15, lines 381-5.

REVIEWER: 2
Page 4. Line 84-85. Is this LDL lowering effect by nuts consistently shown? A reference should be added after that sentence.

AUTHOR REPLY: As requested, we have added a reference in the INTRODUCTION section, under introduction, page 4, lines 82-3.This reference cites studies that show the LDL lowering effect of nuts and emphasizes that the greatest effect is seeing in those individuals with high baseline LDL-C.